

## Main sol. 1092

Sea Salt (SIGMA)	30.00	g
<b>Wolfe's mineral elixir</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	3.00	g
NaHCO <sub>3</sub>	2.50	g
Yeast extract	2.00	g
<b>Wolin's vitamin solution (10x)</b>	2.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, bicarbonate, yeast extract, vitamins and sulfide) and sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic serum vials (e.g., Balch-type tubes) containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Autoclave at **110°C** for 20 min. Add yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.0-7.2.
2. After inoculation pressurize vessels with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 2 bar overpressure.